RAPID, SIMPLE DNA STAINING FOR FIRE ANT EGGS

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Chromosomal DNA in eggs of the fire ant *Solenopsis invicta*, can be demonstrated using the method of Schmuck and Metz (1931. Science 72:600-1), but the procedure is tedious and time-consuming. Eggs are packed in *Drosophila* pupal skins prior to treatment and the staining procedure in itself requires approximately 12 h to complete. In addition, eggs are unavoidably lost. I present technical modifications that simplify their technique, reduce the time required and eliminate egg loss.

Eggs to be stained are placed in a 5 cm length of glass tubing cut from the wide end of a Pasteur pipette. This container is fitted at one end with a square of filter paper, held in place with a Beem's capsule lid (5 mm diameter), through which a hole (4 mm diameter) has been drilled on center (Fig. 1). The apparatus functions as a colander; the cap and filter paper provide for solution drainage. Egg batches are labeled by penciling the information on the filter paper square. These containers are placed in a beaker of appropriate size and the solutions used in the procedure are poured into and pipetted out of the beaker.

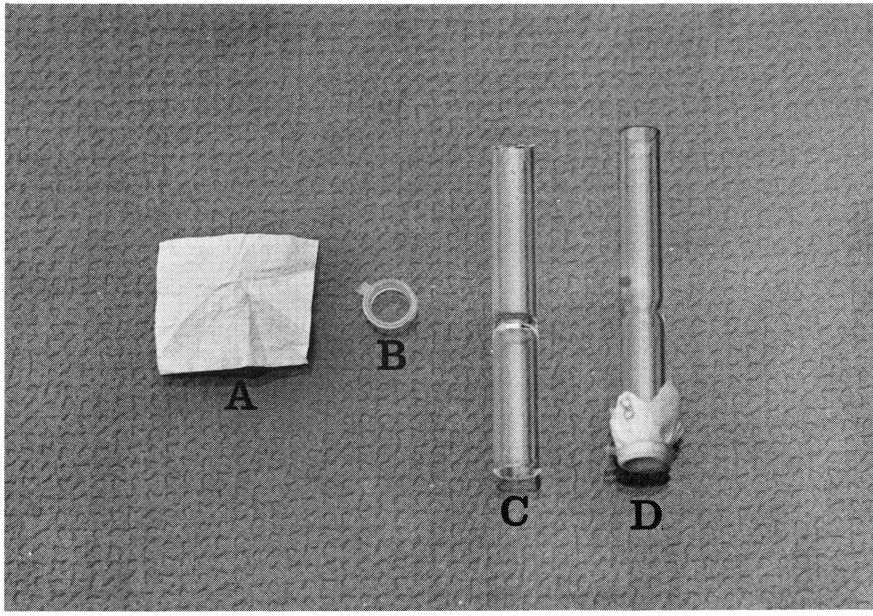

Fig. 1. Apparatus used to hold fire ant eggs during staining. (A — Filter paper square. B — Perforated Beem's capsule lid. C — Tubing cut from Pasteur pipette. D — Assembled apparatus.)

Eggs are fixed for 1 h in a modified Carnoy's solution (equal parts of chloroform, absolute ethanol and glacial acetic acid). This is followed by 4 absolute ethanol changes at 30 min intervals, then ½ h each in 95% and 50%

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ethanol. Eggs can be left overnight in the 95% ethanol and the staining completed the following day. After rinsing with tap water the eggs are hydrolyzed in 2 changes of 5 N HCL for 5 and 60 min at room temperature. Hydrolysis according to these conditions makes the reaction time less critical and yields higher Feulgen values than when hydrolysis is carried out at 60°C using 1 N HCL (Humason, Gretchen. 1979. *Animal Tissue Techniques.* 4th ed. Freeman, San Francisco). Eggs are then bleached for 5 min in a freshly-made sulfurous acid bleaching solution and stained in fuchsin-sulfuric acid Schiff reagent for 1 h. The formulae for these solutions can be found in *The Microtomist's Vade-mecum* (Lee, A. B. 1937. 10th ed. Blakiston, Philadelphia).

Following staining, the eggs are again bleached for 5 min each in 2 changes of the sulfurous acid solution and rinsed in 4 changes of tap water, 2 min each. Dehydration proceeds through an ethanol series, 3 min each in 50%, 2 changes of 95% and 2 changes of 100% ethanol. Eggs are cleared in a 1:1 solution of absolute ethanol and xylene, followed by 2 changes of xylene, each for 3 min.

After the last clearing step, the colander is removed from the beaker and allowed to drain; the eggs are trapped by the filter paper and cap. The filter paper square is then detached from the rest of the apparatus and applied to a slide with Permount. A 22 × 40 mm cover glass is held in place and the rather bulky preparation is flattened with a spring-hinge clothespin. Eggs can be examined using a compound or dissecting microscope. This procedure can be completed in 7 h or less.

While this technique is almost foolproof, there are a few problems that can arise. Eggs may stick to the sides of the container but can easily be dislodged with a probe during dehydration or clearing. Insufficient bleaching and/or rinsing after staining results in residual stain retention by the cytoplasm and pink coloration. This is not a disaster, however, since the more darkly-stained nuclei stand out in contrast. It is also critical to mount the eggs quickly after clearing to prevent the evaporation of the xylene and a resulting whitening of the eggs.

When small numbers of eggs are to be stained, or when it is important to have an ordered mounted sequence of stained eggs, a glass slide replaces the tube carrier. The slide is coated with a thin layer of Mayer's egg albumin and glycerol, just enough to provide an adhesive surface, before applying and arranging the eggs. The slide is then heated in an 80°C oven for 3 min, removed and allowed to cool, and then placed in the fixative. The eggs are fixed in Carnoy's for 30 min, dehydrated in 2 changes of absolute ethanol, 30 min each, and hydrolyzed for only 30 min; otherwise the procedure is the same as outlined. The staining is carried out in glass Petri dishes rather than Coplin jars because keeping the sides in a horizontal position minimizes the possibility of egg loss. This procedure can be completed in approximately 5 h.

Smears can be made using the same general protocol. After the eggs are arranged on the albuminized slide, they are covered with a 2 × 2 cm square of Saran Wrap, coated with vegetable oil on the side touching the eggs. A coverslip is placed on top and the eggs are compressed to the point of membrane rupture. This slide preparation is heated as specified, cooled briefly and placed in the fixative. After about 10 min, the coverslip is removed and the Saran Wrap is gently peeled from the slide. The smear is then carried through the rest of the procedure.

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Slides that have been prepared through the heating step can be frozen and stored at 0°C for subsequent staining. Eggs in the pipette colanders can also be frozen before processing, for months if necessary, without sacrificing the quality of the results.

These techniques have been used to differentiate between embryonated and trophic (non-embryonated) eggs of the fire ant and to examine the early developmental stages of fire ant embryos, but they could probably be applied to a variety of problems concerning the eggs of other species as well. — Susan H. Voss, Department of Entomology, University of Georgia, Athens, Georgia 30602. (Accepted for publication Oct. 8, 1984)

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